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Supporting Information

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Silica Immobilization of an Enzyme via Genetic Engineering of the Diatom *Thalassiosira pseudonana*

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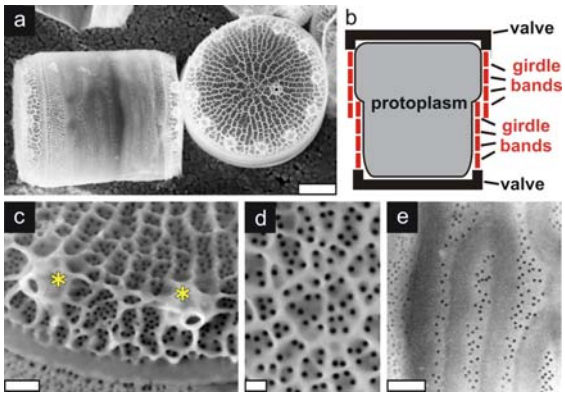

Figure S1. Structure of *T. pseudonana* silica. Images in (a) and (c-e) were obtained by scanning electron microscopy. a) Intact biosilica structures from two different cells, one in "girdle view" (left) and the other in "valve view" (right). b) Schematic of a cross section through a diatom cell. c) Detail of the rim area of the valve. The asterisks indicate two tube like-structures (rimoportulae). Each valve contains about 9-12 rimoportulae that are regularly spaced along the valve rim (see right cell wall in image a). d) Detail of the valve surface close to the center. e) Detail from the girdle band region. Bars: 1 µm (a), 200 nm (c, e), 100 nm (d).

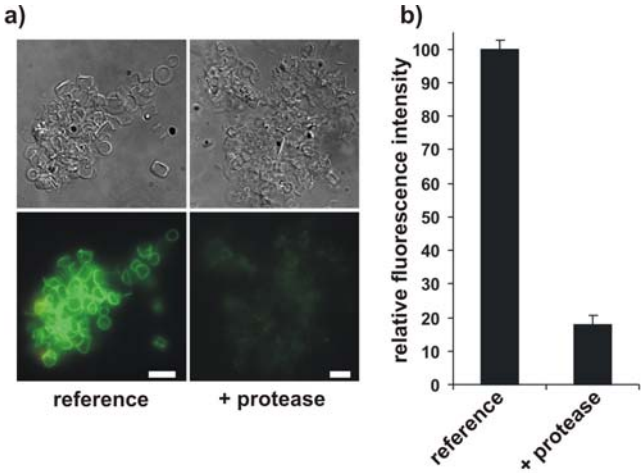

Figure S2. Accessibility of biosilica immobilized GFP. Silica isolated from *T. pseudonana* cells expressing TpfcpSil3GFP was incubated in 100 mM Tris-HCl pH 8.0 for 4 hours at 37 ^{o}C in the absence (reference) or presence (+ protease) of 0.1 mg·ml^{-1} pronase. a) Micrographs of biosilica taken from the same object with normal light (top) and with epifluorescence (bottom) using the same exposure time. b) Fluorescence intensities of the same amounts of tpSil3-GFP containing biosilica from reference and protease treated samples (determined by fluorophotometry).

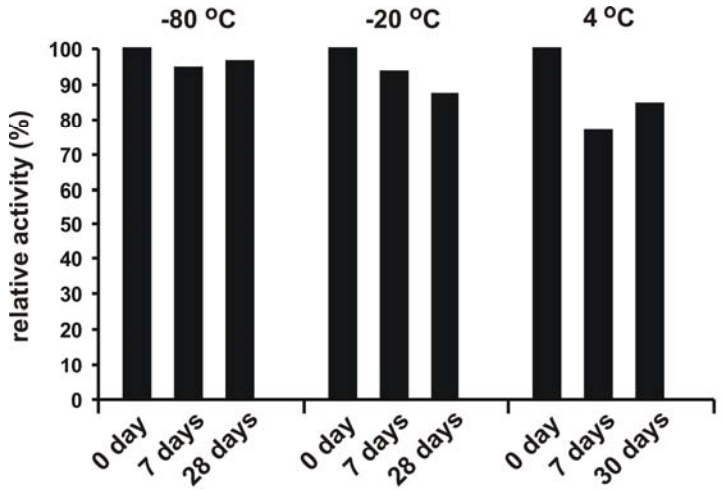

Figure S3. Long-term storage stability of diatom silica immobilized HabB. The material was stored at the indicated temperatures, equal aliquots were withdrawn at the indicated days and tested for HabB activity. Activities were normalized to the activity at the beginning of the experiment (0 day).

Experimental Section

Scanning electron microscopy (SEM). Isolated *T. pseudonana* biosilica was mounted onto copper grids and air dried. Images were obtained with a LEO 1530 field emission scanning electron microscope (Oberkochen, Germany).

Fluorescence Microscopy. Confocal fluorescence microscopy imaging was performed using an inverted Zeiss LSM 510 laser scanning microscope (Jena, Germany). GFP fluorescence (Argon laser, 488 nm) was detected using a 505/550-nm bandpass filter. For dual imaging, chloroplast auto-fluorescence (HeNe laser, 543 nm) was detected with a 585 nm long pass filter in the multitrack mode of the microscope. For photobleaching experiments fluorescence images were recorded using a Zeiss Axiovert 200 microscope (Carl Zeiss AG, Jena, Germany), equipped with a Piston GFP bandpass filter set (Ex: HQ470/40x, Dichroic Q495LP, Em: HQ515/30M; Chroma Technology Corp., Rockingham, VT) and a photometrics CoolSNAP HQ camera (Photometrics, Tucson, AZ). ImageMaster2.1 software (Photon Technology International, Birmingham, NJ) was used to capture at 5 or 10 sec intervals still images of cells that were exposed to constant UV irradiation (Mercury Short ARC HBO lamp, 103W). ImageJ software was used to determine in each image the fluorescence intensity in a selected 9 μm^2 sized area of the cell.

Fluorophotometry. A Shimadzu RF-5301PC spectrofluorophotometer was used for quantification of fluorescence in biosilica samples. The excitation wavelength was 485 nm, the emission maximum was 510 nm, and the slit width at both wavelengths was 5 nm.

Construction of *T. pseudonana* expression vectors. The fusion gene encoding tpSil3-GFP was generated by PCR using the gene splicing by overlap extension method (SOEing), which introduced the amino acid sequence G-G-E-F between the tpSil3 domain[3] and the GFP domain[4]. The PCR product was cloned into the EcoRV and NotI sites of *T. pseudonana* expression vector pTpfcp generating plasmid pTpfcp/*sil3-gfp*, which contains the promoter and terminator sequence of a *T. pseudonana fcp* gene.[1] For biosilica incorporation of HabB a generic pTpfcp-based expression vector (pTfcp/*sil3*$_{nt}$) was constructed that enabled insertion of DNA fragments downstream of the *tpSil3* gene by restriction cloning. The HabB gene was amplified by PCR and inserted into the EcoRV site of pTfcp/*sil3*$_{nt}$.

pTpfcp/*sil3-gfp*: A plasmid containing the *egfp* gene fused to the 3'-end of the *tpSil3* gene has previously been constructed by GeneSOEing, introducing the sequence 5'-GGTGGCGAATTC-3' (encodes peptide G-G-E-F) between the two genes (unpublished result). The plasmid was used as a template for PCR using sense primer 5'-ACCAAAATGAAGACTTCTGCCATTG-3' and antisense primer 5'-GAAT**GCGGCCGC**TTACTTGTACAGCTCGTC-3', which amplified the complete *tpSil3-egfp* fusion gene and introduced a NotI restriction site at the 3'-end (bold). The resulting 1184-bp PCR product was digested with NotI and incorporated into the EcoRV and NotI sites of vector pTpfcp[1], generating pTpfcp/*sil3-gfp*.

pTpfcp/*sil3*$_{nt}$: This is a generic vector for targeting proteins to the *T. pseudonana* biosilica. It contains the *tpSil3* gene that was amplified by PCR using sense primer 5'-ACCAAAATGA AGACTTCTGCCATTG-3' and antisense primer 5'-ATC**GCGGCCGC***GCATGC*GATATCTC CACCAGCGCTCATGGAGTGGACC-3', which introduced NotI (bold), SphI (italics) and EcoRV (underlined) restriction sites. The resulting 1169 bp PCR fragment was digested with NotI and incorporated in to the EcoRV and NotI sites of pTpfcp[1] generating the plasmid pTpfcp/*sil3*$_{nt}$, which contains a short multiple cloning site (EcoRV, SphI, NotI) following the 3'-end of the *tpSil3* gene.

pTpfcp/*sil3-habB*: The HabB gene[2] was amplified by PCR using primers 5'-ATGACGCTGCACACTCCC-3' (sense) and 5'- TCACGCGGC CGGCTGCGG-3' (antisense). The resulting 495 bp PCR product was incorporated into the EcoRV site of vector pTpfcp/*sil3* generating plasmid pTpfcp/*sil3-habB*.

The sequences of all PCR products were confirmed by DNA sequencing. The transformation of *T. pseudonana* and selection of transformants was performed as described previously.[1]

References

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2. J. K. Davis, G. C. Paoli, Z. He, J. N. Lloyd, C. C. Sommerville, J. Spain, *Appl. Environ. Microbiol*. **2000**, 66, 2965-2971.
3. N. Poulsen. N. Kröger, *J. Biol. Chem*. **2004**, 279, 42993-42999.
4. G. H. Patterson, S. M. Knobel, W. D. Sharif, S. R. Kain, D. W. Piston, *Biophys. J.* **1997**, 73, 2782-2790